DESIGN AND IN VITRO EVALUATION OF COLON TARGETED DRUG DELIVERY SYSTEM USING TAMARIND SEED POLYSACCHARIDE AND EUDRAGIT S100 AS CARRIERS

Dissertation work submitted to

THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY, CHENNAI

In partial fulfillment of the award of degree of

MASTER OF PHARMACY

(Pharmaceutics)

Submitted by

HARI KRISHNAN.P

Under the guidance of

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March 2009

COLLEGE OF PHARMACY

SRI RAMAKRISHNA INSTITUTE OF PARAMEDICAL SCIENCES

COIMBATORE – 641044

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Certificate

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Date:

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P Harikrishnan.

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INTRODUCTION

Historically, the pharmaceutical industry has always maintained and enjoyed strong financial earnings and business growth. Such healthy growth is not only made possible by launching new products but also by life-cycle management of older, existing products, which is playing an increasingly significant role. This aspect becomes even more important once the revenue drops significantly from the existing molecule due to patent protection expiry.

In the area of targeted delivery, the colonic region of the GI tract is the one that has been embraced by scientists and is being extensively investigated over the past two decades. Targeted delivery to the colon is being explored not only for local colonic pathologies, thus avoiding systemic effects of drugs or inconvenient and painful trans-colonic administration of drugs, but also for systemic delivery of drugs like proteins and peptides, which are otherwise degraded and or poorly absorbed in the stomach and small intestine but may be better absorbed from the more benign environment of the colon. This is also a potential site for the treatment of diseases sensitive to circadian rhythms such as asthma, angina and arthritis. Furthermore, there is urgent need for delivery to the colon of drugs that are reported to be absorbable in the colon, such as steroids, which would increase efficiency and enable reduction of the

required effective dose. The treatment of disorders of the large intestine, such as irritable bowel syndrome (IBS), colitis, crohn's disease and colon disease, where it is necessary to attain a high concentration of the active agent, maybe efficiently achieved by colon-specific delivery.[1]

The oral route is considered to be most convenient for administration of drugs to patients. Oral administration of conventional dosage forms normally dissolves in the stomach fluid or intestinal fluid and absorb from these regions of the GIT depends upon the physicochemical properties of the drug. It is a serious drawback in conditions where localized delivery of the drugs in the colon is required or in conditions where a drug needs to be protected from the hostile environment of upper GIT. Dosage forms that deliver drugs into the colon rather than upper GIT proffers number of advantages. Oral delivery of drugs to the colon is valuable in the treatment of diseases of colon (ulcerative colitis, crohn's disease, carcinomas and infections) there by high local concentration can be achieved while minimizing side effects that occur because of release of drugs in the upper GIT or unnecessary systemic absorption. The colon is rich in lymphoid tissue, uptake of antigens into the mast cells of the colonic mucosa produces rapid local production of antibodies and this helps in efficient vaccine delivery. The colon is attracting interest as a site where poorly absorbed drug molecule may have an improved bioavailability. This region of the colon is recognized as having a somewhat less hostile

environment with less diversity and intensity of activity than the stomach and small intestine. Additionally, the colon has a longer retention time and appears highly responsive to agents that enhance the absorption of poorly absorbed drugs. Apart from retarding or targeting dosage forms, a reliable colonic drug delivery could also be an important starting position for the colonic absorption of perorally applied, undigested, unchanged and fully active peptide drugs. As the large intestine is relatively free of peptidases such special delivery systems will have a fair chance to get their drug sufficiently absorbed after peroral application. The simplest method for targeting of drugs to the colon is to obtain slower release rates or longer release periods by the application of thicker layers of conventional enteric coatings or extremely slow releasing matrices.[2]

The colon, as a site for drug delivery, offers distinct advantages on account of a near neutral pH, a much longer transit time, relatively low proteolytic enzyme activity, and a much greater responsiveness to absorption enhancers. These criteria favour this distal part of the gastrointestinal tract (GIT) as a site for the delivery of various drug molecules, including proteins and peptides. Colon-specific delivery systems should prevent the release of the drug in the upper-part of GIT and require a triggering mechanism to affect an abrupt release on reaching the colon. In the past, various primary approaches for colon-specific delivery, such as pro-drugs, pH sensitive polymers, timed release

delivery systems, and microbially degraded delivery systems, have achieved limited success. The majority of these systems developed during the past decade, were based on pH and time dependent mechanisms with limited in-vivo evaluation. Minor variation in pH between the small intestine and the colon makes the pH-dependent systems less specific, in terms of targeted release in the colon. Time-dependent formulations predominantly depend on the transit time of the delivery system in the GIT. A major limitation with these systems is that *in vivo* variation of the small intestinal transit time may lead to release of the bioactive in the small intestine or terminal part of the colon. The pathophysiological state of an individual will have a significant impact on the performance of these time-dependent systems. Patients with irritable bowel syndrome and ulcerative colitis exhibited accelerated transit through different regions of the colon. [3] Relevant drugs for colon-specific associated diseases are listed in table 1 and colon-specific associated diseases are presented diagrammatically in figure1.

Fig. 1: Diagrammatical presentation of Colon associated diseases[3]

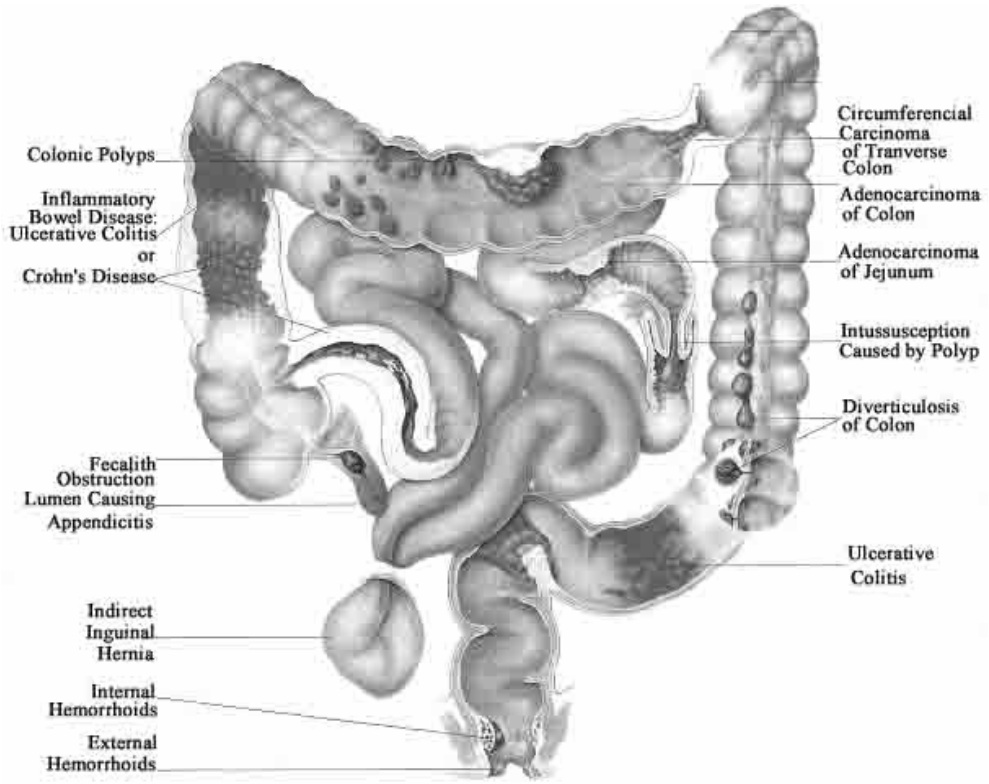


Table 1: Relevant drugs for colon-specific associated diseases[3]

Target Sites	Disease Conditions	Symptoms	Drugs and active agents
Topical /Local action	Inflammatory Bowel Disease (Crohn's disease)	Diarrhea, Abdominal pain and cramping, blood in stool, ulcers, reduced appetite and weight loss	Hydrocortisone, Budenoside, Prednisolone, Sulfasalazine
	Ulcerative colitis	Inflammation in the rectum, rectal bleeding, rectal pain	5-Amino salicylic acid, Sulfasalazine, Balsalazide, and mercaptopurine
	Irritable bowel syndrome	Abdominal pain or cramping, a bloated feeling, flatulence, diarrhea or constipation — people with IBS may also experience alternating bouts of constipation and diarrhea, mucus in the stool	Dicyclomine, Hyoscine, Propantheline, Cimetropium, Alosetron, Tegaserod
	Colorectal cancer	A change in bowel habits, narrow stools, rectal bleeding or blood in stool, persistent abdominal discomfort, such as cramps, gas or pain, abdominal pain with a bowel movement, unexplained weight loss	5-Flourouracil, Leucovorin,and cetuximab
	Diverticulitis	Formation of pouches (diverticula) on the outside of the colon due to bacterial infection	Bactrim,Flagyl, Sulfatrim, Metronidazole
	Antibiotic associated colitis	Overgrowth of Clostridium difficile and its subsequent toxin production	clindamycin, broad-spectrum penicillins (e.g., ampicillin, amoxicillin), and cephalosporins
	Hirschsprung's disease	Severe form of constipation in which bowel movement occurs only once or twice a week	Metronidazole, Vancomycin, Loperamide,Botulinum toxin
Systemic action	Ulcerative colitis	Ulcerative proctitis, pancolitis, fulminant colitis	Prednisolone metasulfobenzoate, tixocortol pivalate, fluticasone propionate, beclomethasone

Colon-specific drug delivery has been approached by a number of methods exploiting changes in the physiological parameters along the gastrointestinal tract. The GIT transit time was utilized to formulate colon specific drug delivery systems which are so designed that their drug release is delayed to the time required for transiting drug from mouth cavity to distal part of small intestine i.e., ileum and subsequently drug release in the colon. Factors influencing the transit time of pharmaceutical dosage forms in the various regions of the gastrointestinal tract appear to depend on diet, gastrointestinal motility and physical activity of the person, fasted or fed state of the person. The change in pH along the gastrointestinal tract was also used to develop colon specific drug delivery systems by applying coatings that were intact at low pH and dissolved at neutral pH. The pH of the colon is however; often lower than the pH of the small intestine, which in turn may be as high as 8 or 9, resulting in a too early release of a drug.

A number of specific regional characters of the colon can be explored for site specific drug delivery to the colon. In the colon, an extensive growth of anaerobic micro organisms is observed. These colonic microflora produce a large number of hydrolytic as well as reductive enzymes which can potentially be utilized for colon-specific drug delivery. Prodrugs and coatings based on azo aromatic polymers and matrices containing azo aromatic cross-links are examples of systems that are

potentially degradable by reductive enzymes released by colonic bacteria. Apart from azo reductase enzyme release other polysaccharidases like glucosidases; glycosidases are also released by colonic microflora, which are responsible for the degradation of polysaccharides. Hence drug delivery systems based on polysaccharide can also be used for colon specific drug delivery.[3]

FORMULATION TECHNOLOGIES FOR COLON SPECIFIC DRUG DELIVERY

Delivery systems for targeted delivery in the GIT could be categorized into four categories: (i) pH-based systems, (ii) Time-based delivery systems, (iii) Pressure-based systems, and (iv) Enzyme-based systems (prodrugs/coatings and matrices).

pH-controlled drug delivery systems

Use of pH-dependent polymers is based on the difference in pH-levels along the GIT. The polymers described as pH-dependent in colon specific delivery are insoluble at low pH levels but become increasingly soluble as pH rises. The pH in the GIT varies between and within individuals and also between healthy and patients which could lead to the failure of the system in the treatment of inflammatory bowel diseases. Moreover, during acute stage of inflammatory bowel disease colonic pH has been found to be significantly lower than the physiological pH. It must be also taken into consideration that, between the terminal ileum and the

distal colon, there is a slightly acidic region in the proximal colon, due to the fermentation of poly and oligosaccharides to short-chain fatty acids, which might affect drug release profiles and the reproducibility of drug release. Most commonly used pH-dependent coatings polymers are copolymers of methacrylic acid and methyl methacrylate containing carboxyl groups Eudragit S100 which is soluble above pH 7 and Eudragit L above pH 6 are mostly used polymers in targeted drug delivery to the colon. Eudragit S coatings have been used to target the anti-inflammatory drug 5-aminosalicylic acid in single-unit formulations on the colon.[1]

Recently, a new type of delivery system has been developed to delivery drug to the colon for the treatment of ulcerative colitis. EUDRACOL™ which is a combined pH- and time-based multi-unit dosage form which is already available for targeting drug to the colon. EUDRACOL™ consists of 5-aminosalicylic acid containing core which is then first coated with an aqueous dispersion of Eudragit RL: RS (2:8) and with a new pH-dependent anionic polymer Eudragit FS. The latter dissolves rapidly at pH above 7, triggering the onset of drug release in distal GIT. Eudragit RL/RS produce a slow release of drug from the pellets. The performance of this newly designed drug delivery system has been investigated in vitro as well as *in vivo*, and compared with solely pH-dependent system.[4]

Time controlled drug delivery systems

Usually, time-dependent drug delivery systems are designed to deliver drugs after a lag of five to six hours. This approach is based upon the theory that the lag time equates to the time taken for the dosage form to reach the colon. The lag time is dependent on size of dosage form and gastric motility associated with the pathological condition of the individual. The residence times can vary from a few seconds to a number of hours. On the other hand the small intestine transit time is reported to be more consistent at three to four hours. Since the system is unable to sense and adapt to an individual's condition, the approach clearly limits the utility. An example of such a dosage form would be an impermeable capsule body containing the drug, fitted with a hydrogel plug that is used to deliver the drug after a predetermined time. This dosage form, for example Pulsincap releases the drug once the hydrogel plug hydrates and swells in aqueous media and is ejected from the device, thereby allowing the release of the drug from the capsule.[1]

Systems based on time controlled release are identified as unsuitable for drug delivery in the colon for the treatment of inflammatory bowel diseases. The rationale behind all time-release delivery systems is valid provided that small intestine transit times remain constant. Changes in GI tract motility can significantly affect time-release drug delivery systems targeting the release of drugs to the colon. [4]

Pressure controlled drug delivery systems

This novel delivery mechanism is utilised to initiate the release of the drug in the distal part of the gut. The muscular contractions of the gut wall generate pressure, which is responsible for grinding and propulsion of the intestinal contents**.** Pressure-sensitive drug formulations release the drug as soon as a certain pressure limit is exceeded. Polymers used for this topic form firm layers that are destroyed by an increase of the luminal pressure in the colon caused by peristaltic waves. Disintegration of a pressure-controlled drug delivery system that consists of a gelatin capsule with an inner ethylcellulose coating is triggered by peristaltic waves, destroying the ethylcellulose film. As water ingresses into the core, the low substituted hydroxypropylcellulose will start swelling. The cap which is made of the water-insoluble ethylcellulose (EC) cannot persist the swelling pressure. The ethylcellulose cap disintegrates releasing the active drug from the container within the capsule. The most important factor for disintegration of the formulation is the thickness of the water-insoluble ethylcellulose film. [4]

Bacterially triggered drug delivery systems

The use of GI microflora as a mechanism of drug release in the colonic region has been of great interest to researchers in recent times. The colonic microflora produces a variety of enzymes that are not present or different from those in the stomach and the small intestine and could

therefore be used to deliver drugs to the colon after enzymatic cleavage of degradable formulation components or drug carrier bonds.[1] Most of the bacteria in the colon are anaerobic (95%) and facultative aerobic (5%). More than 400 bacterial species have been found in colon able to ferment complex polysaccharides. The carbohydrates are fermented into short chain fatty acids, carbon dioxide, hydrogen, methane and other products by the enzymes glycosidase and polysaccharidase. This concept could be divided into (i) the use of prodrugs breakdown by bacterial enzymes within the colon and (ii) use of tools (coatings/matrices) susceptible to colonic bacteria. [4]

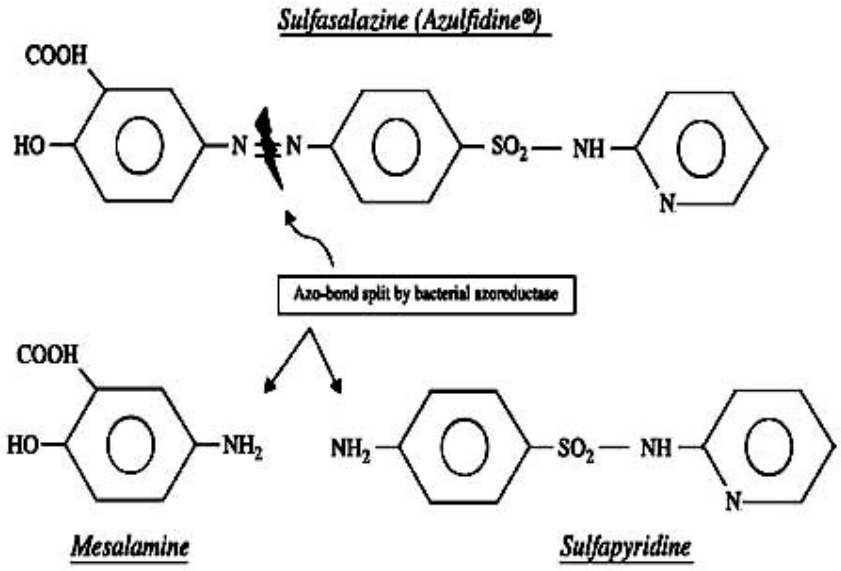
Prodrugs

A prodrug is a pharmacologically inactive derivative of a parent molecule that requires some form of transformation *in vivo* to release the active drug at the target site. This approach involves covalent linkage between the drug and its carrier in such a manner that upon oral administration the moiety remains intact in the stomach and small intestine. The type of linkage that is formed between the drug and carrier would decide the triggering mechanism for the release of the drug in the colon. This biotransformation is carried out by a variety of enzymes, mainly of bacterial origin, present in the colon. The enzymes that are mainly targeted for colon drug delivery include azoreductase, β - galactosidase, β-xylosidase, nitroreductase, glycosidase deaminase etc. [1]

Generally, a prodrug is successful as a colon drug carrier if it is hydrophilic and bulky, to minimise absorption from the upper GI tract and, once in the colon, it is converted into a more lipophilic drug molecule that is then available for absorption. [4]

Certain drugs can be conjugated to different sugar moieties to form glycosides. The use of these azo compounds for colon targeting has been in the form of hydrogels as a coating material for coating the drug cores and as prodrugs. Sulphasalazine, which was used for the treatment of rheumatoid arthritis, was later known to have potential in the treatment of inflammatory bowel disease (IBD). This compound has an azo bond between 5-ASA and sulphapyridine. The azo bond split into mesalamine and sulfapyridine by bacterial azo reductase.[2]

Fig 2: Mechanism of microbial activation of sulfasalazine in the colon[4]



Coatings and matrices

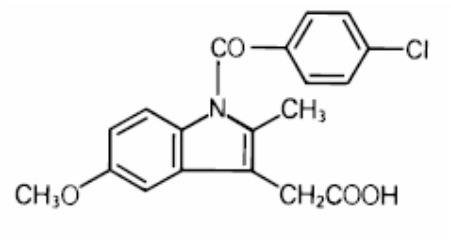
Polysaccharide-based formulations represent a relatively simple formulation concept because of its safety (most can be used without additional safety testing) if there are no chemical modifications to the polysaccharide. Moreover, polysaccharides are inexpensive and readily available in a variety of structures with a variety of properties. They can be easily modified chemically and bio chemically and are highly stable, safe, non-toxic, hydrophilic and gel forming and in addition biodegradable, which suggests their use in targeted drug delivery systems to the colon. Due to the high hydrophilicity polysaccharides possess high solubility and swelling in aqueous medium which lead to premature drug release in the upper GIT

when using polysaccharides solely as coating materials for colon drug delivery systems. To control the high swelling of polysaccharides hydrophobic polymers should be added in order to reduce the swelling, and subsequently to ensure that no or very low drug is released until it reaches the colon [4]

The polysaccharides naturally occurring in plant (e.g., pectin, guar gum, inulin), animal (e.g.,chitosan, chondroitin sulphate), algal (e.g., alginates) or microbial (e.g., dextran) origin were studied for colon targeting. [2]

PROFILE OF DRUG EMPLOYED IN THE STUDY

INDOMETHACIN [10-13]



Chemistry

1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H- Indole-3-acetic acid

Category

Anti-inflammatory, Analgesic

Description

White to pale yellow, crystalline powder, odourless

Molecular weight

Indomethacin has a molecular weight of 357.79 g/mol

Empirical formula

$C_{19}H_{16}NO_4Cl$

Solubility

Soluble in chloroform; slightly soluble in ethanol (95%) and practically insoluble in water

Storage

Stored in well closed, light resistant containers

PHARMACODYNAMICS

Mechanism of action

Indomethacin has prominent anti-inflammatory and analgesic-antipyretic properties similar to those of the salicylates. Indomethacin is a more potent inhibitor of the cyclooxygenases than that of aspirin, but patient intolerance generally limits its use to short-term dosing. Indomethacin has analgesic properties distinct from its anti-inflammatory effects, and there is evidence for central and peripheral actions.

Pharmacokinetics and metabolism

Oral Indomethacin has excellent bioavailability. Peak concentrations occur 1 to 2 hours after dosing. Indomethacin is 90% bound to plasma proteins and tissues. The concentration of the drug in the CSF is low, but its concentration in synovial fluid is equal to that in plasma within 5 hours of administration. Between 10% and 20% of indomethacin is excreted unchanged in the urine, partly by tubular secretion. The majority is converted to inactive metabolites.

Half life

The half-life in plasma is variable, perhaps because of enterohepatic cycling, but averages about 2.5 hours.

Therapeutic uses

- Indomethacin is effective for relieving joint pain, swelling, and tenderness, increasing grip strength, and decreasing the duration of morning stiffness.
- It is estimated to be approximately 20 times more potent than aspirin.
- It suppresses inflammation in a manner similar to steroids, but less side effects of sedation.
- They are widely used for the treatment of inflammatory disorders and painful conditions such as rheumatoid arthritis, gout, bursitis, painful menstruation, and headache.

Drug Interactions

Indomethacin does not directly modify the effect of warfarin, but platelet inhibition and gastric irritation increase the risk of bleeding; concurrent administration is not recommended. Indomethacin antagonizes the natriuretic and antihypertensive effects of furosemide and thiazide diuretics and blunts the antihypertensive effect of b receptor antagonists, AT_1 receptor antagonists, and ACE inhibitors.

Adverse Effects

A very high percentage (35% to 50%) of patients receiving usual therapeutic doses of indomethacin experience untoward symptoms, and about 20% must discontinue its use because of the side effects. Most adverse effects are dose-related.

Gastrointestinal complaints are common and can be serious. Diarrhea may occur and sometimes is associated with ulcerative lesions of the bowel. Underlying peptic ulcer disease is a contraindication to Indomethacin use. Acute pancreatitis has been reported, as have rare, but potentially fatal, cases of hepatitis.

PROFILES OF POLYMERS EMPLOYED IN THE STUDY

In the present study Tamarind seed polysaccharide (TSP), Guar gum, Pectin and Eudragit S100 were used in the preparations of matrix tablets.

Tamarind seed polysaccharide (TSP) [14-16]

Synonyms

Tamarind seed polyose, Tamarind gum

Molecular weight

TSP is a polymer with an average molecular weight of 52,350g/mol

Structural formula

It is a galactoxyloglucan mainly of three sugars—glucose, galactose, and xylose—in a molar ratio of 3:1:2.

Description

Tamarind gum or Tamarind Seed Polysaccharides (TSP) or hydrocolloid is a polysaccharide polymer (D-galactose, D-xylose and D-glucose) obtained from endosperm of kernels of Tamarind seeds. Purified TSP is a high-molecular-weight, non-ionic, neutral, branched polysaccharide consisting of a cellulose-like backbone that carries xylose and galactoxylose substituents. Polysaccharides extracted from Tamarind seeds are known to produce gels in presence of sugar over a wide pH

range. TSP is an interesting candidate for pharmaceutical use, e.g. as a carrier of a variety of drugs for controlled release.

Solubility

It is insoluble in organic solvents and dispersible in hot water to form a highly viscous gel such as a mucilaginous solution with a broad pH tolerance and adhesivity.

Safety

It is safe to use in food and pharmaceutical formulations as it is a plant origin.

Pharmaceutical applications

Recently important properties of TSP have been identified. They include noncarcinogenicity, mucoadhesivity, biocompatibility and high drug holding capacity. These led to its application as excipient in hydrophilic drug delivery system.

- TSP is an interesting candidate for pharmaceutical use, as a carrier of a variety of drugs for controlled release applications.
- It is used as binder in tablets, gelling agent, thickening agent, as emulsifier and as stabilizer in food, and pharmaceutical industries.
- As a carrier polysaccharide

Guar gum [17, 18]

Non proprietary names

BP : Guar galactomannan

PhEur : Guar galactomammanum

USPNF : Guar gum

Synonyms

E412; Galactorol; Guar flour; Jaguar gum; Mey prodor; Mey proguar

Chemical name and CAS Registry number

Galactomannan polysaccharide [9000-30-0]

Empirical formula

$(C_6H_{12}O_6)_n$

Molecular weight

Guar gum has a molecular weight of 220, 000 g/mol

Structural formula

Guar gum consists of linear chains of (1-4)-β-D-Manno-pyranosyl units with α-D-galactopyranosyl units attached by (1-6) linkages and at ratio 1:2.

Description

The USPNF 20 describes Guar gum as a gum obtained from the ground endosperms of *Cyamopsis tetragonolobus*. It consists chiefly of high molecular weight hydro colloidal polysaccharide, composed of

galactan and mannan units combined through glycosidal linkages which may be described chemically as galactomannan

The main components are polysaccharide composed of D-galactose and D-mannose in molecular ratio of 1:4 to 1:2. Guar gum occurs as an odourless, white to yellowish- white powder with a bland taste.

Solubility

It is practically insoluble in organic solvents. In cold or hot water, Guar gum disperses and swells almost immediately to form highly viscous colloidal dispersion.

Safety

Guar gum is widely used in foods and oral and topical pharmaceutical formulations. Excessive consumption may cause gastro intestinal disturbance such as flatulence, diarrhoea, nausea.

Pharmaceutical applications

- Guar gum is a galactomannan commonly used in cosmetics, food products and pharmaceutical formulations.
- It has also been investigated in the preparation of sustained-release matrix tablets in the place of cellulose derivatives.
- It has also been examined for use in colonic delivery.

Pectin[19]

Synonyms

Genu Pectin L 200, citrus pectin, Apple pectin, Colyer pectin, Mexpectin, Methyl pectin

Chemical name and CAS Registry number

Pectin [9000-69-5]

Empirical formula

$(C_6H_{10}O_7)_n$

Molecular weight

Pectin has a molecular weight of typically 60–130,000 g/mol.

Structural formula

The characteristic structure of pectin is a linear chain of α-(1-4)-linked D-galacturonic acid that forms the pectin-backbone, a homogalacturonan.

Description

Pectin is a complex mixture of polysaccharides that makes up about one third of the cell wall dry substance of higher plants. Much smaller proportions of these substances are found in the cell walls of grasses. The highest concentrations of pectin are found in the middle lamella of cell wall, with a gradual decrease as one passes through the primary wall toward the plasma membrane. Although pectin occurs commonly in most of the plant tissues, the number of sources that may be used for the

commercial manufacture of pectins is very limited. Pectin is a long chain of pectic acid and pectinic acid molecules.

Solubility

Pectins are soluble in pure water. Monovalent cation (alkali metal) salts of pectinic and pectic acids are usually soluble in water; di- and trivalent cations salts are weakly soluble or insoluble.

Safety

Pectins are widely used in food and pharmaceutical formulations as it is a plant origin.

Pharmaceutical applications

- Pectin has a promising pharmaceutical uses and is presently considered as a carrier material in colon-specific drug delivery systems.
- Pectin is an interesting candidate for pharmaceutical use, e.g. as a carrier of a variety of drugs for controlled release applications.
- Many techniques have been used to manufacture the pectin-based delivery systems, especially ionotropic gelation and gel coating.

Eudragit S100 [20]

Non proprietary names

BP : Methacrylic acid – methyl methacrylate copolymer

USPNF : Methacrylic acid polymer

Synonyms

Eudragit; kollicot MAE 30 D; kollicot MAE DP; polymeric methacrylates

Chemical names and CAS Registry number

Poly (methacrylic acid, methylmethacrylate) 1:2 [25086-15-1]

Molecular weight

Copolymer of methacrylic acid and methyl methacrylate having a mean molecular mass about 135,000 g/mol

Description

Methacrylic acid copolymers are anionic copolymerisation products of methacrylic acid and methyl methacrylate. The ratio of free carbonyl groups to the ester is approximately 1:2. It is readily soluble in neutral to weakly alkaline conditions and form salts with alkalis , thus affording film costs that are resistant to gastric media , but soluble in intestinal fluid. It is a white free flowing powder with at least 95% of dry polymer

Solubility

Soluble in acetone and alcohols, insoluble in dichloromethane and petroleum ether

Safety

It is generally regarded as non toxic and non irritant material. A daily in take of 2 mg /kg body weight of Eudragit S100 (equivalent to approximately 150 mg for an average adult) may be regarded as essentially safe in humans

Applications in pharmaceutical formulations

- Polymethacrylates are primarily used in oral capsules and tablet formulations as film coating agent.
- It is mainly used for enteric coating for solid dosage forms
- It is used as a binder in both aqueous and organic wet granulation process
- It may be used in direct compression process in quantities of 10-50%.

REVIEW OF LITERATURE

Savur et al[14] (1947) investigated the nature of the polysaccharide fraction of tamarind seed which show how it differs from pectins, a conclusion necessitating revision of the views held to account for many of the phenomena of pectin-sugar-acid jelly formation. They compared tamarind seed polysaccharide with fruit pectins by studying the sugar-acid jelly formation and various other characters.

Taylor et al[24] (1985) examined samples of the xyloglucan polysaccharide from tamarind seed using X-ray diffraction. Periodicities indexing on a spacing of 2.06 nm were observed along the chain direction. This value was twice that reported for cellulose and is commensurate with four β-1,4- linked glucose residues. Flat, ribbon-like two-fold helical models for the β-1,4- linked polyglucose backbone, with two possible schemes for decoration with xylose and galactose side groups, were proposed.

Hartemink *et al*[21] (1996) studied Fermentation of Xyloglucan, a cell wall polysaccharide and part of the dietary fiber fraction of the diet, that can be degraded by the intestinal microflora. Selective elimination of bacterial groups from faecal slurries indicated that clostridia are the main group of intestinal bacteria responsible for degradation of xyloglucan polymer. In addition to the polymer-degrading strains, some other strains

were able to degrade and ferment oligosaccharides produced from tamarind seed.

Kirilmaz et al[33] (1996) studied the release of Oxolamine citrate matrix tablets prepared with direct compression and wet granulation using Eudragit RS PM as polymer. The matrix tablets were prepared at drug polymer ratio (1:0.5, 1:0.75, 1:1, 1:1.5, 1:2). Drug polymer was found to be effective on the release of Oxolamine citrate from matrix tablets. The matrix tablets prepared with direct compression technique released drug faster than matrix tablets prepared by wet granulation. It was observed that release rate of Oxolamine citrate can be decreased by heating the matrix tablets prepared by both direct compression and wet granulation

Vandamme *et al*[6] (2001) reviewed the use of polysaccharides to target drugs to colon to improve the specificity of drug release. Certain types of polysaccharides can be used to create the dosage forms. These excipients are specially degraded by the colonic microflora and have been used for polymer drug conjugates, coatings and matrixing agents. To prevent the premature release, these are combined with hydrophobic polymers. Authors reviewed the potential uses of polysaccharides, the limits and future developments in this field with natural polymers.

Sumathi et al[23] (2002) studied the sustained release behaviour of both water-soluble (acetaminophen, caffeine, theophylline and salicylic acid) and water insoluble (indomethacin) drugs from tamarind seed

polysaccharide isolated from tamarind kernel powder and the effect of incorporation of diluents like microcrystalline cellulose and lactose on release of caffeine and partial cross-linking of the polysaccharide on release of acetaminophen. The insoluble drug showed near case II or zero order release mechanism. The rate of release was in the decreasing order of caffeine, acetaminophen, theophylline, salicylic acid and indomethacin. An increase in release kinetics of drug was observed on blending with diluents.

Raghavan *et al*[38] (2002) prepared and evaluated colon specific drug delivery systems based on polysaccharides locust bean gum and chitosan in the ratio of 2: 3, 3: 2 and 4: 1 using *in vitro* and *in vivo* methods. The *in vitro* studies in pH 6.8 phosphate buffer containing 2% w/v rat caecal contents showed that the cumulative percentage release of mesalazine after 26 h were 31.25±0.56, 46.25±0.96, 97.5±0.26 (mean±S.D.), respectively. The *in vivo* studies conducted in nine healthy male human volunteers for the various formulations revealed that, the drug release was initiated only after 5 h (*i.e.*) transit time of small intestine and the bioavailability of the drug was found to be 85.24±0.10, 196.08±0.12, 498.62±0.10 µg h/ml 26 (mean±S.D.), respectively. These studies on the polysaccharides demonstrated that the combination of locust bean gum and chitosan as a coating material proved capable of protecting the core tablet containing mesalazine during the condition mimicking mouth to

colon transit with a better dissolution profile, higher bioavailability and hence a potential carrier for drug targeting to colon.

Sinha et al[22] (2003) compared on the usual enteric coating polymers viz. Eudragit, a cellulose acetate phthalate with shellac and ethyl cellulose, as carriers for colon specific drug delivery. Lactose based Indomethacin tablets are prepared. These are coated with one of the coating polymers to a varying coat thickness. The coated formulations are evaluated for dissolution rates under simulated stomach and small intestine conditions. From the dissolution data obtained, it was found that the dissolution rate varied with the type and concentration of the polymer applied. Comparative dissolution data revealed that, of all the various polymers and coat thickness used, a 3% (m/m) coat of shellac was most suitable for colonic drug delivery.

Chourasia et al[2] (2003) reviewed various pharmaceutical approaches to colon drug delivery systems. The various strategies for targeting orally administered drugs to the colon include covalent linkage of a drug with a carrier, coating with pH-sensitive polymers, formulation of timed released systems, exploitation of carriers that are degraded specifically by colonic bacteria, bio adhesive systems and osmotic controlled drug delivery systems. Various prodrugs (sulfasalazine, ipsalazine, balsalazine and olsalazine) have been developed that are aimed to deliver 5-amino salicylic acid (5-ASA) for localized chemotherapy

of inflammatory bowl disease (IBD). Microbially degradable polymers especially azo cross linked polymers have been investigated for use in targeting of drugs to colon. Certain plant polysaccharides such as amylose, inulin, pectin and guar gum remains unaffected in the presence of gastrointestinal enzymes and pave the way for the formulation of colon targeted drug delivery systems.

Tabandeh *et al*[25] (2003) developed a sustained-release tablet formulation having proper release profile insensitive to moderate changes in tablet hardness that is usually encountered in manufacturing. In this study, matrix aspirin (acetylsalicylic acid) tablets with ethylcellulose (EC), Eudragit RS100 (RS), and Eudragit S100 (S) were prepared by direct compression. The release behaviors were then studied in two counterpart series of tablets with hardness difference of three Kp units, and compared by non-linear regression analysis. The release pattern for both the S-containing and RS-containing formulations fitted best in Higuchi model, and the proper equations were suggested. In the EC-containing formulation, Higuchi and also zero-order models were probable models for the release, and a combination equation for the release was suggested. In the S-containing formulation, the release profile was completely sensitive to the hardness change. In RS-containing series, the slope of the release graph did not change due to the hardness decrease, but the y-intercept or the lag time in release was decreased. In EC-containing matrix tablets,

both the slopes and the y-intercepts did not change by the decrease in hardness. In conclusion, EC with an amount as little as 10 percent in formulation could make sustained-release aspirin tablets in which the release profile is not sensitive to moderate changes in hardness.

Momin et al [26] (2004) developed colon targeted drug delivery systems for sennosides using guar gum as a carrier. Matrix tablets containing various proportions of guar gum (30%-40%) were prepared by wet granulation technique using starch paste as a binder. The tablets were evaluated for content uniformity and *in vitro* drug release study as per BP method. Guar gum matrix tablets released 4-18% sennosides in the physiological environment of gastrointestinal tract depending on the proportion of the guar gum used in the formulation. The matrix tablets containing 50% of guar gum were found to be suitable for targeting of sennosides for local action in the colon.

Friend[7] (2004) studied on new oral delivery systems for treatment of inflammatory bowel disease. Targeted delivery systems for treatment of IBD are designed to increase local tissue concentrations of anti-inflammatory drugs from lower doses compared with systemic administration. Delivery systems reviewed rely on temporal control, changes in pH along the GIT, the action of local enzymes to trigger drug release, and changes in intraluminal pressure. Dissolution of enteric polymer coatings due to a change in local pH and reduction of azo-bonds

to release an active agent are both used in commercially marketed products. Newer approaches showing promise in treating IBD are based on polysaccharides.

Chaurasia *et al*[39] (2006) prepared guar gum microspheres containing methotrexate characterized for local release of drug in the colon, which is a prerequisite for the effective treatment of colorectal cancer. Guar gum micros pheres were prepared by the emulsification method using glutaraldehyde as a cross-linking agent. The *in vitro* drug release was investigated using a US Pharmacopeia paddle type (type II) dissolution rate test apparatus in different media (phosphate-buffered saline [PBS], gastrointestinal fluid of different pH, and rat cecal content release medium). The drug release in PBS (pH 7.4) and simulated gastric fluids followed a similar pattern and had a similar release rate, while a significant increase in percent cumulative drug release (91.0%) was observed in the medium containing rat cecal content. Guar gum microspheres showed adequate potential in achieving local release of drug *in vitro* release studies.

Alebiowu *et al* [14] (2006) studied the influence of Polymer Particle size on the release of indomethacin from tamarind seed polyose. TSP was used to prepare sustained-release formulations of indomethacin (IND), an anti-inflammatory drug substance with a short half-life of 2.4 ± 0.4 h. Its dosage regimen is 25 mg thrice daily, with peptic ulceration as

its major side effect. IND is a suitable candidate for a sustained-release formulation. Tablets were prepared using direct compression because a wet granulation method would cause an initial swelling of the polymer, thereby affecting the swelling profile of the various fractions during release testing of the matrix tablets.

Singh[4] (2006) mentioned that solid formulations intended for targeted drug release into the lower gastrointestinal (GI) tract are beneficial for the localized treatment of several diseases and conditions, mainly inflammatory bowel diseases, irritable bowel syndrome and colon cancer. This study reviewed on the recent patent literature concerning various modified-release (MR) formulation technologies that are claimed to provide colonic delivery for a wide array of therapeutic molecules. These technologies either utilize a single or a combination of two or more physiological characteristics of the colon, which includes pH, microflora (enterobacteria), transit time, and luminal pressure.

Ghaffari *et al* [27] (2006) prepared and evaluated theophylline pellets with Eudragit RS aqueous dispersions, containing various amounts of pectin-chitosan complex and different coating mass gains, using a fluidized-bed apparatus. Pellets containing theophylline as a model drug and microcrystalline cellulose, in a ratio of 6:4, were prepared by the extrusion-spheronization method. Twelve formulations were developed, which differed in two factors: coating mass gain (10, 15 and 20%, m/m)

and the amount of pectin-chitosan complex (5, 10, 15 and 20%, m/m). Twelve formulations were developed, which differed in two factors: coating mass gain and the amount of pectin-chitosan complex. Drug release studies were conducted using the USP apparatus I (basket) in dissolution media, mimicking the conditions prevailing in the stomach, small intestine or colon. In formulations with15 or 20% (m/m) of coating mass gain and 5 or 10% (m/m) of pectin-chitosan, the burst drug release was eliminated and replaced by the lag phase of drug release. In the case of burst drug release in the colonic medium, formulations with 20% (m/m) of coating mass gain and 15 or 20%(m/m) of pectin-chitosan were found to be better than the other formulations.

Jain *et al* [3] (2007) studied the ability to deliver drugs to the human colon using bio degradable polymers and a final overview of the various approaches to target drugs to the colon utilizing natural polysaccharides, the limits and the future developments in this field with these natural polymers and . To deliver the compounds in a non-degraded form to the lower part of the gastrointestinal tract, they must first of all pass through the stomach, the upper part of the intestine and must use the characteristics of the colon to specifically release the drugs in this part of the digestive tract. Polysaccharidases are bacterial enzymes that are available in sufficient quantity in colon to be exploited in colon targeting.

Nasra *et al* [29] (2007) studied on potential of matrix, multilayer and compression coated tablets of metronidazole to reach the colon intact has been investigated *in vitro*, using pectin as a carrier. Matrix tablets containing various proportions of pectin were prepared by wet granulation and direct compression techniques. Multilayer tablets were formulated using pectin as release controlling layers, on either side of metronidazole matrix tablets. Rapidly disintegrating metronidazole core tablets were prepared and compression coated with pectin. The effect of the coat: core ratio as well as the incorporation of different percentages of chitosan in the pectin coat on drug release was investigated. *In vitro* release studies indicated that matrix and multilayer tablets failed to control the drug release in the physiological environment of stomach and small intestine. On the other hand, compression coated formulations were able to protect the tablet cores from premature drug release, but at high pectin coat: core ratios 4: 1 (F13) and 5: 1 (F14). Inclusion of chitosan 3% and 5% w/w (F12) in the pectin coat offered better protection at a lower coat: core ratio (3: 1).

Siepmann *et al* [9] (2007) mentioned in his review that the use of polymer blends as coating materials for controlled drug delivery systems can offer major advantages, including: (i) facilitated adjustment of desired drug release patterns, mechanical properties and drug release mechanisms, (ii) improved film formation and storage stability, and (iii) the

possibility to develop novel strategies for site specific drug delivery within the gastro intestinal tract (e.g., colon targeting). For instance, the blended polymers can be incompatible, aqueous polymer dispersions might flocculate and plasticizers potentially redistribute from one polymer into the other during curing and or long term storage. The author gave an overview on the current state of the art of the use of polymer blends as coating materials for controlled drug delivery, explaining the major advantages and potential pitfalls.

Huanbutta *et al*[31] (2008) studied the factors affecting preparations of chitosan microcapsules for colonic drug delivery. Chitosan microcores containing diclofenac sodium coated with Eudragit S100 were prepared by a desolvation technique. Factors affecting morphology, particle size and zeta potential of microcapsules was evaluated, i.e. weight ratio of DS: CS: ED, surfactant (polysorbate80), anti-adherent (silicon dioxide), and the use of sonication or homogenization in preparation processes. Adding of polysorbate 80 could not reduce the size of microcapsules; but silicon dioxide could reduce the size and aggregation of microcapsules. Finally, the use of sonication and homogenization was effective in reducing of the size of microcapsules to 53.45±0.63 and 58.72±1.28 micrometers, respectively.

Ravi *et al*[32] (2008) developed a novel colon targeted tablet formulation using pectin as carrier and Diltiazem HCl and Indomethacin as model drugs. The tablets were coated with inulin followed by shellac and were evaluated for average weight, hardness and coat thickness. *In vitro* release studies for prepared tablets were carried out for 2 h in pH 1.2 HCl buffer, 3 h in pH 7.4 phosphate buffer and 6 h in simulated colonic fluid. The drug release from the coated systems was monitored using UV/Vis spectroscopy. *In vitro* studies revealed that the tablets coated with inulin and shellac have limited the drug release in stomach and small intestinal environment and released maximum amount of drug in the colonic environment. The study revealed that polysaccharides as carriers and inulin and shellac as a coating material can be used effectively for colon targeting of both water soluble and insoluble drugs.

Patel *et al*[34] (2008) prepared and evaluated multiparticulate system combining pH-sensitive property and specific biodegradability for colon targeted delivery of 5-fluorouracil (5-FU). Study was to prepare and evaluate the colon-specific alginate beads of 5-FU for the treatment of colon cancer. Calcium alginate beads were prepared by extruding 5-FU loaded alginate solution to calcium chloride solution, and gelled spheres were formed instantaneously by ionotropic gelation reaction using different ratios of FU and alginate, alginate and calcium chloride, stirring speeds (500-1500 rpm), and reaction time. The core beads were coated with

Eudragit S-100 to prevent drug release in the stomach and provide controlled dissolution of enteric coat in the small intestine and maximum drug release in the colon. Morphology and surface characteristics of the formulation were determined by scanning electron microscopy. *In vitro* drug release studies were performed in conditions simulating stomach to colon transit. No significant release was observed at acidic pH, however, when it reached the pH where Eudragit S-100 starts to dissolve, drug release was observed.

Rao *et al*[28] (2003) studied the potential use of pectin in combination with two added hydrocolloids, i.e., hydroxy-propyl-methyl cellulose and hydroxyethyl cellulose in varied concentrations and coated with ethyl cellulose and cellulose acetate phthalate. The results of *in vitro* drug release showed that the matrix tablets prepared with pectin, hydroxyethyl cellulose (20 percent) when coated with ethyl cellulose and cellulose acetate phthalate were found to be 63.0 percent, 8.4 percent, and 4.5 percent, respectively, in after eight hours during drug release study period. These results were confirmed with the results of roentgenographic studies in nine healthy human volunteers to find the shape and integrity of the dosage form. The X-ray photographs revealed that the enteric-coated tablet was visible only up to 5.5 hours and at the end of eighth hour, the photograph has not shown any presence of tablet indicating the loss of shape and size by the microflora present in the colon region. So, the

results of *in vitro* and roentgenographic studies revealed that pectin, hydroxy ethyl cellulose (20 percent) base coated with ethyl cellulose and cellulose acetatephthalate was found to be a promising carrier for naproxen to colon.

SCOPE AND OBJECTIVE OF THE STUDY

The site specific delivery of drugs to the colon has implications in number of therapeutic areas, which include topical treatment of Crohn's disease, Ulcerative colitis, constipation, colorectal cancer, spastic colon and irritable bowl syndrome. A colonic delivery system would be additionally being valuable when delay in absorption is therapeutically desirable in treatment of diseases like rheumatoid arthritis, which are influenced by circadian rhythm. These diseases are known to have peak symptoms when awakening from night time sleep. [7]

Various approaches available for colon specific drug delivery include (a) coating with pH dependent systems(b) design of timed release dosage forms (c) the use of carrier that are degraded exclusively by colonic bacteria. The pH dependant systems are designed to release the drug to above a particular pH of GIT. The poor site specificity of pH dependant systems, because of large variation in the pH of the GIT was very well established. The timed release systems release their load after a predetermined time period of administration. The site specificity of these systems is considered poor because of large variations in gastric emptying time and passage across the ileocaecal junction. The best alternative approach for colon specific drug delivery is the use of carriers that are degraded exclusively by colonic bacteria. The micro flora of colon is in the

range of 10^{10}-10^{12} (CFu/ml) consisting mainly of anaerobic bacteria, e.g. Bacteroids, *Eubacteria*, *Clostridia enterococci*, *Enterobacteria* and *Ruminococcus* etc. This vast micro flora fulfils its energy need by fermenting various types of substrates that have been left undigested in the small intestine e.g. di and tri sacchrides, polysaccharides etc. For this fermentation micro flora produces a vast number of enzymes like β-glucoronidase, β-xylosidase α-arabinosidase, β-galactosidase, nitro reductase, azo reductase, deaminase and urea dehydroxylase. Because of the presence of the biodegradable enzymes only in the colon, the use of the biodegradable polymers for colon specific drug delivery systems is considered to be a more site- specific approach as compared to other approaches. [8]

Several studies are published on the use of naturally obtained polysaccharides as a carrier in colon targeted drug delivery systems. The different polysaccharides that have been evaluated as a carrier for colonic drug delivery include pectin[30], chitosan[27], guar gum[26], locust bean gum[38], inulin and alginates.[34]

Xyloglucan, a cell wall polysaccharide and part of the dietary fiber fraction of the diet, can be degraded by intestinal microflora. Clostridia are the main group of intestinal bacteria responsible for degradation of xyloglucan polymer. It is proposed that xyloglucan is degraded in vivo by endo-β-glucanases produced mainly by clostridia, followed by fermentation

of the oligosaccharides by a larger group of bacteria. In tamarind seeds how ever, xyloglucan is the predominant polysaccharide. [21]

Although Tamarind seed polysaccharide (TSP) is used as an ingredient in food material and in pharmaceuticals, it has not been evaluated as carrier for colon targeted drug delivery system. TSP is a galactoxyloglucan isolated from seed kernel of *Tamarindus indica.* [21]

Indomethacin has been selected as a suitable candidate for this study because it is used to treat rheumatoid arthritis, a disease which is influenced by circadian rhythm. The disease shows peak symptoms while awakening from night time sleep. Therefore a delay in drug release is essential in case of Indomethacin.[12]

Studies by Patel and Amol[34] have reported the use of a combination of natural polysaccharide and pH dependant system as a best choice of carrier for targeting drugs to the colon.

The present investigation is aimed at evaluating suitability of a combination of naturally occurring polysaccharide obtained from *Tamarindus indica* seed and Eudragit S100 as a carrier for colon targeted drug delivery system.

EXPERIMENTAL WORK

MATERIALS USED

- Indomethacin

  Gift sample from Microlabs Pvt Ltd, Banglore, India

- Guar gum

  Hi media Laboratories, Mumbai

- Pectin

  Hi media Laboratories, Mumbai

- Eudragit S100

  Roehm Pharma Polymers, Darmstadt, Germany

- Petroleum ether

  Qualigens Fine Chemicals, Lucknow

- Diethyl ether

  Qualigens Fine Chemicals, Lucknow

- Starch

  Hi media Laboratories, Mumbai

- Lactose

  S.d Fine Chemicals, Mumbai

- Methanol

  Qualigens Fine Chemicals, Lucknow

INSTRUMENTS AND EQUIPMENTS USED

- UV Visible spectrophotometer

  Jasco V-530 double beam

- Dissolution test apparatus USP

  Tab Machines, Mumbai

- Tablet compression machine

  Rimek mini press-1

Table 2: Formulation codes for Indomethacin matrix tablets

Formulation code	Polymer concentration (%)
G1F1	TSP(30)+Eudragit S100(30)
G1F2	TSP(40)+Eudragit S100(30)
G1F3	TSP(50)+Eudragit S100(30)
G1F4	TSP(60)+Eudragit S100(30)
G1F5	TSP(70)+Eudragit S100(30)
G2	Guar gum(50)+Eudragit S100(30)
G3	Guar gum(50)+pectin(35)+Eudragit S100(30)

METHODS

Isolation of Tamarind Seed Polysaccharide (TSP) [23]

Tamarind seed polysaccharide was prepared following methods by Rao *et al*., in three batches on a laboratory scale. To 20g of tamarind kernel powder, 200ml of cold distilled water was added and slurry was prepared. The slurry was poured into 800ml of boiling distilled water. The solution was boiled for 20 minutes under stirring condition in a water bath. The resulting thin clear solution was kept overnight so that most of the proteins and fibers settled out. The solution was then centrifuged at 5000 rpm for 20 minutes. The supernatant was separated and poured into twice the volume of absolute ethanol by continuous stirring. The product was pressed between felt. The precipitate was washed with absolute ethanol, diethyl ether and petroleum ether and then dried at 50-60°C under vacuum. The dried material was ground and sieved to obtain granules of different particle size range.[23]

Characterization of TSP by X-ray diffraction[24]

Diffraction pattern of powdered TSP sample was recorded With a Bruker AXS D8 Advance X ray diffractometer. (STIC/SF/2008-09/1813).

PREPARATION OF INDOMETHACIN MATRIX TABLETS CONTAINING TSP AND EUDRAGIT S100 AS CARRIERS

Matrix tablets of Indomethacin were prepared by wet granulation method. Lactose was used as a diluent and a mixture of talc and magnesium stearate was added as lubricant. Tamarind seed polysaccharide (TSP) (50%) and Eudragit S100 (30%) was included in formulations.[25] The composition of the matrix formulations used in the study containing 25 mg of Indomethacin is shown in table 3.

TSP was sieved separately and mixed with Indomethacin and lactose. Powders were blended and granulated using 10% starch paste. The wet mass was passed through mesh (10#) and granules were dried at 50°C for 30 minutes. The dried granules were passed through mesh (20#) and these granules were lubricated with mixture of magnesium stearate and talc (2:1). The lubricated granules were compressed using round, flat and plain punches using Rimek mini press -1 machine. [26]

Matrix formulations of Indomethacin with varying concentrations of TSP ranging from 30% to 70% along with Eudragit S 100 (30%) also prepared in different batches in order to identify the formulation with the best release profile. Composition of these matrix formulations are given in table 3.

Table 3: Composition of matrix tablets of Indomethacin with varying concentrations of TSP ranging from 30% to 70% along with Eudragit S100 (30%)

Ingredients	Quantity (mg) present in each tablet				
	G1F1	G1F2	G1F3	G1F4	G1F5
Indomethacin	25	25	25	25	25
TSP	69.5	95	120	145	192
Eudragit S100	69.5	69.5	69.5	75	82
Lactose	80	54.5	29.5	5	5
Talc	4	4	4	4	4
Magnesium stearate	2	2	2	2	2
Total weight	250	250	250	256	310

PREPARATION OF INDOMETHACIN MATRIX TABLETS CONTAINING GUAR GUM+EUDRAGIT S100 AND GUAR GUM+PECTIN+EUDRAGIT S100 AS CARRIERS

The formulation with the best drug release profile identified from the above batch were then compared with the drug release profile of Indomethacin matrix tablets prepared using a) Guar gum +Eudragit S100 and b) Guar gum+ Pectin+Eudragit S100.[30] Composition of these matrix formulations are shown in table 4 and 5

Table 4: Composition of matrix tablet of Indomethacin containing Guar gum (50%) and Eudragit S100 (30%)

Ingredients	Quantity (mg) present in tablet
Indomethacin	25
Eudragit S100	69.5
Guar gum	120
Lactose	23.5
Talc	4
Magnesium stearate	2
Total weight	250

Table 5: composition of matrix tablet of Indomethacin containing Guar gum (50%), Pectin (35%) and Eudragit S 100(30%)

Ingredients	Quantity (mg) present in tablet
Indomethacin	25
Eudragit S100	60
Guar gum	120
Pectin	74
Lactose	5
Talc	4
Magnesium stearate	2
Total weight	290

EVALUATION OF MATRIX TABLETS [36]

Hardness and friability of the matrix tablets formulated were evaluated using a Pfizer hardness tester and a Roche friabilator respectively. For friability, test 20 tablets were taken and weight was determined. Then they were placed in friabilator and allowed to make 100 revolutions. Tablets were then dedusted and reweighed. To test hardness of tablets, 5 tablets were taken from each batch and subjected to test. Disintegration time of the tablets was determined in tablet disintegration test machine.

Determination of drug content [11]

Indomethacin matrix tablets were tested for their drug content by Indian pharmacopeia method. A quantity of powdered contents of 10 tablets equivalent to 50 mg of Indomethacin was weighed accurately and10 ml of water was added and allowed to stand for 10 minutes, with occasional shaking. 75 ml methanol was added, shaken well, and added sufficient methanol to produce 100 ml and then filtered. To 5 ml filtrate sufficient mixtures of equal volumes of methanol and 7.2 phosphate buffer was added to produce 100 ml. Absorbance was measured at 318 nm. Drug content of Indomethacin was calculated.

STANDARD GRAPH OF INDOMETHACIN

Preparation of stock solution

100 mg of Indomethacin is dissolved in 100ml of ethanol to get a concentration of 1000 µg/ml.

From this 10 ml was taken and made up to 100 ml with ethanol to get a concentration of solution 100 µg/ml.

Preparation of various concentrations of drug solution

1. 1 ml of stock solution was made up to 10 ml with ethanol to give 10 µg/ml
2. 2 ml of stock solution was made up to 10 ml with ethanol to give 20µg/ml
3. 3 ml of stock solution was made up to 10 ml with ethanol to give 30 µg/ml
4. 4 ml of stock solution was made up to 10 ml with ethanol to give 40 µg/ml
5. 5 ml of stock solution was made up to 10 ml with ethanol to give 50 µg/ml

Procedure

Various concentrations of Indomethacin (10, 20, 30, 40 and 50 µg/ml) were prepared as mentioned above. Absorbance of the solution was measured against reagent blank at 318 nm using UV spectrophotometer. A standard graph between concentrations vs. absorbance was plotted. A straight line passing through origin was obtained.

PREPARATION OF BUFFER SOLUTIONS [37]

Hydrochloric acid (0.1N)

8.5 ml of concentrated Hydrochloric acid was added to 1000 ml volumetric flask and made up to the volume with distilled water.

Phosphate buffer pH 7.4

50 ml of 0.2 M Potassium dihydrogen phosphate is taken in a 200 ml of volumetric flask and 39.1 ml of 0.2 M Sodium hydroxide was added and made up to the volume with distilled water.

Phosphate buffer pH 6.8

50 ml of 0.2 M Potassium dihydrogen phosphate was taken in a 200 ml of volumetric flask and 22.4 ml of 0.2 M Sodium hydroxide was added and made up to the volume with distilled water.

IN VITRO DRUG RELEASE STUDIES [26-32]

The ability of matrix tablets of Indomethcin (containing TSP and Eudragit S100 as carriers) to remain intact in the physiological environment of stomach and small intestine was assessed by mimicking mouth to colon transit. Drug release studies were carried out using USP XXIII dissolution apparatus (100 rpm, 37± 0.5°C) in 900 ml 0.1N Hydrochloric acid for 2 hours as the average gastric emptying time is ~2 h. The dissolution medium was replaced with 900ml pH 7.4 phosphate buffer and tested for 3 hours as the average small intestinal transit time is about 3 hours. The dissolution medium was then replaced with pH 6.8 phosphate buffer and drug release studies were carried out for 24 hours as the usual colonic transit time is 20-30 hours. 10 ml of sample was taken at the end of specified time intervals filtered and filtrate was analysed for Indomethacin at 318nm using UV spectrophotometer. A 10 ml volume of filtered, fresh dissolution medium was added to make the volume after each sample withdrawal. [26]

Matrix formulations of Indomethacin with varying concentrations of TSP ranging from 30% to 70% along with Eudragit S100 (30%) were prepared in different batches in order to identify the formulation with the best release profile. Composition of these matrix formulations are given in table 2. The formulation with the best drug release profile identified from the above batch were then compared with the drug release profile of

Indomethacin matrix tablets prepared using a)Guar gum(50%)+Eudragit S100 (30%)and b)Guar gum(50%)+Pectin(35%)+Eudragit S100(30%). Composition of these matrix formulations are shown in table 3 and 4. The above procedure was followed in order to carryout the drug release studies for these formulations.

STATISTICAL ANALYSIS

The mean percentage of Indomethacin released from TSP (50%)+Eudragit S 100(30%) matrix formulation in pH 6.8 phosphate buffered solution was compared with that of drug released from formulations containing a)Guar gum+Eudragit and b)Guar gum+Pectin+Eudragit. Paired t test was used to find the statistical significance. P value < 0.05 is considered statistically significant.

RESULTS AND DISCUSSION

X-ray diffraction analysis – x-ray diffraction pattern (figure 3) of TSP did not show any characteristic peak, which indicate that the structure is completely amorphous. The result confers with the x-ray diffraction study of Tamarind seed polysaccharide by Sumathi et al.[23] The result show that the isolated polysaccharide has similar behaviour with that reported by others. This isolated polysaccharide along with Eudragit S100 was used in preparation of matrix tablets of Indomethacin.

The present study was aimed at developing and evaluating matrix tablet of Indomethacin for colon targeting using a combination of Tamarind seed polysaccharide and Eudragit S100 as a matrixing agent. The tablet formulation released decreased amount of Indomethacin in physiological environment of stomach and small intestine. Majority of the drug was released in pH 6.8 phosphate buffer, which is the pH prevailing in the colon.

Since TSP was found to have poor flow characteristics and was to be incorporated in large amounts in matrix tablets Indomethacin tablets were prepared by wet granulation method using 10% starch paste as binder. The diameter and thickness of tablets were 6.5mm and 2mm respectively. The result of hardness test, friability, disintegration test and drug content shown in table 7.

Chapter 7 Results & Discussion

The matrix tablets were subjected to *in vitro* drug release study in 0.1 N HCl (2hours), pH 7.4 phosphate buffer saline (3 hours) and pH 6.8 phosphate buffer saline (24 hours). All the formulations showed no drug release in first 2 hours in stimulated gastric environment (0.1N HCl). The cumulative percentage drug release in pH 7.4 buffer is shown in table 8 to 10 and figure 5 and 6.

Table 11 to 13 and figure 7 and 8 illustrate the cumulative percentage drug release of all matrix formulations in pH 6.8 buffer.

From figure 6 it was clear that small quantity of drug (G1F3 - 28.72%, G2 - 22.13%, G3 - 30.1%) was released in pH 7.4 and majority of drug was released in pH 6.8 PBS buffer.

Tablets of G1F3 released 82.35 % of Indomethacin in pH 6.8 buffer solution; G2 released 75.95%; G3 released 87.11% at 24 hours.

On comparing formulation G1F3 (TSP 50%+ Eudragit S100 30%) with G2 (Guargum 50%+ Eudragit S100 30%) using Paired t- test, the difference in the amount of drug released after 24 hours of dissolution study was not statistically significant, while a statistically significant difference in the amount of drug released was observed on comparing G1F3 (TSP50% + Eudragit S10030%) with G3 (Guar gum 50% + Pectin 35% + Eudragit S100 30%).

Chapter 7 Results & Discussion

The present study revealed that a combination of Tamarind seed polysaccharide and Eudragit S100 may be considered as a potential candidate for targeting drugs to colon. But further studies are warranted using rat caecal contents as TSP undergoes degradation in colon by colonic bacteria. Further in vivo studies using healthy human volunteers may be required in order to establish various pharmacokinetic parameters of Indomethacin matrix tablets containing TSP (50%) and Eudragit S100 (30%).

Fig. 3: X-ray diffraction pattern of Tamarind seed polysaccharide

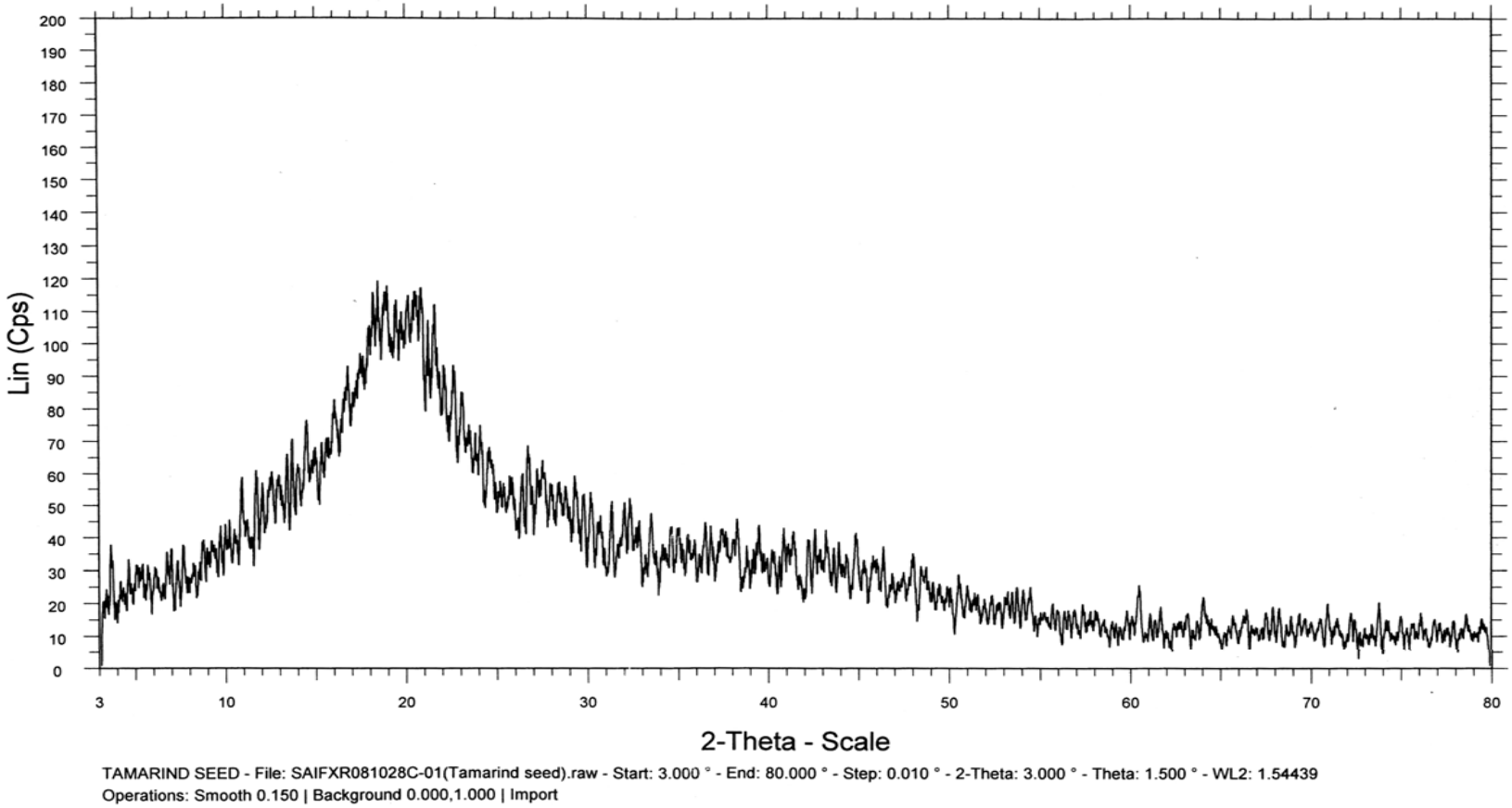


Table 6: Calibration Data for the estimation of Indomethacin (10-50 µg/ml)

S. No.	Concentration in µg/ml	Absorbance at 318 nm
1	0	0.000
2	10	0.2630
3	20	0.4600
4	30	0.6500
5	40	0.8754
6	50	1.1338

Fig. 4: Calibration curve for estimation of Indomethacin (10-50 µg/ml)

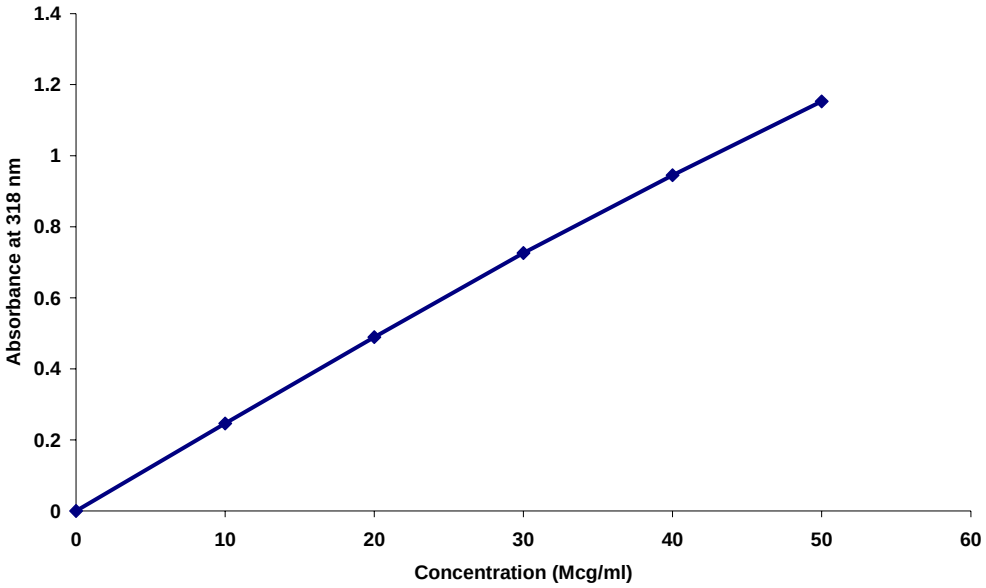


Table 7: Evaluation of Indomethacin matrix tablets

Evaluation of tablets	G1F1	G1F2	G1F3	G1F4	G1F5	G2	G3
Crushing strength (kg/cm^2)	4.6	5.1	5.3	4.9	5.1	4.7	5.2
Friability (%)	0.39	0.32	0.45	0.43	0.39	0.46	0.42
Drug content (%)	103	101	102	103	101	98	102
Disintegration (min)	18	15	15	18	14	16	16

***In vitro* Drug Release Studies**

Table 8: Cumulative percent drug release from Indomethacin tablets containing various concentrations of TSP (30- 70%) + Eudragit S100 (30%) in pH 7.4 buffer

Time in minutes	Percentage release of Indomethacin (%)				
	G1F1	G1F2	G1F3	G1F4	G1F5
30	6.30	8.63	12.3	5.88	4.10
60	11.98	13.68	15.84	9.38	7.94
90	15.10	17.70	18.92	13.56	10.12
120	17.68	19.34	22.64	15.57	12.51
150	18.56	22.82	25.62	16.98	13.83
180	24.12	26.36	28.72	20.12	15.68

Fig. 5: Comparison of release profiles of Indomethacin from matrix formulations containing varying concentrations of TSP (30-50%) + Eudragit S100 (30%) in pH 7.4 phosphate buffer

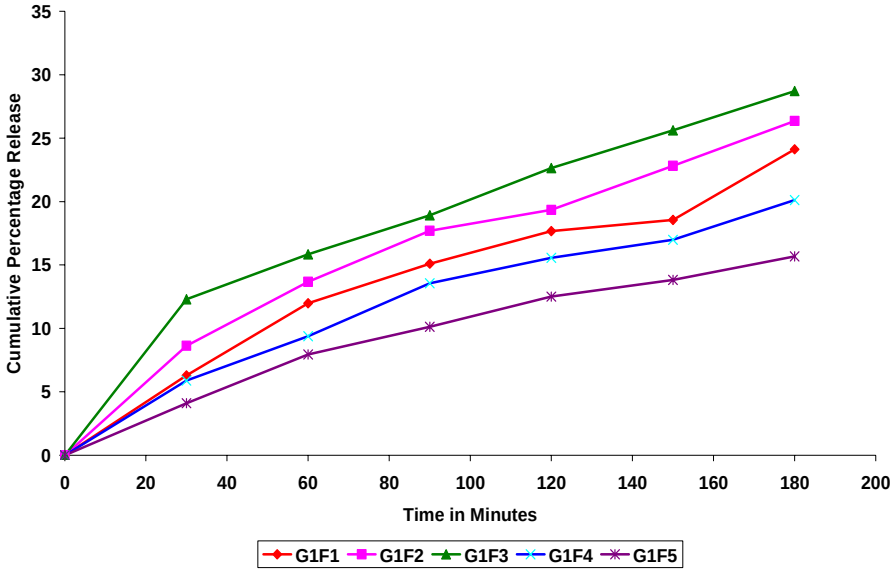


Table 9: Cumulative percent drug release from Indomethacin tablets containing Guar gum (50 %) + Eudragit S100 (30%) in pH 7.4 Buffer

Time in minutes	Percentage release of Indomethacin from G2 (%)
30	11.56
60	12.60
90	13.2
120	13.7
150	14.56
180	22.13

Table 10: Cumulative percent drug release from Indomethacin tablets containing Guar gum (50%) + Pectin (35%) + Eudragit S100 (30%) in pH 7.4 Buffer

Time in minutes	Percentage release of indomethacin from G3 (%)
30	15.20
60	18.63
90	23.40
120	25.45
150	28.56
180	30.10

Fig 6: Comparison of release profiles of Indomethacin from matrix formulations G2, G3, and G1F3 in pH 7.4 phosphate buffer

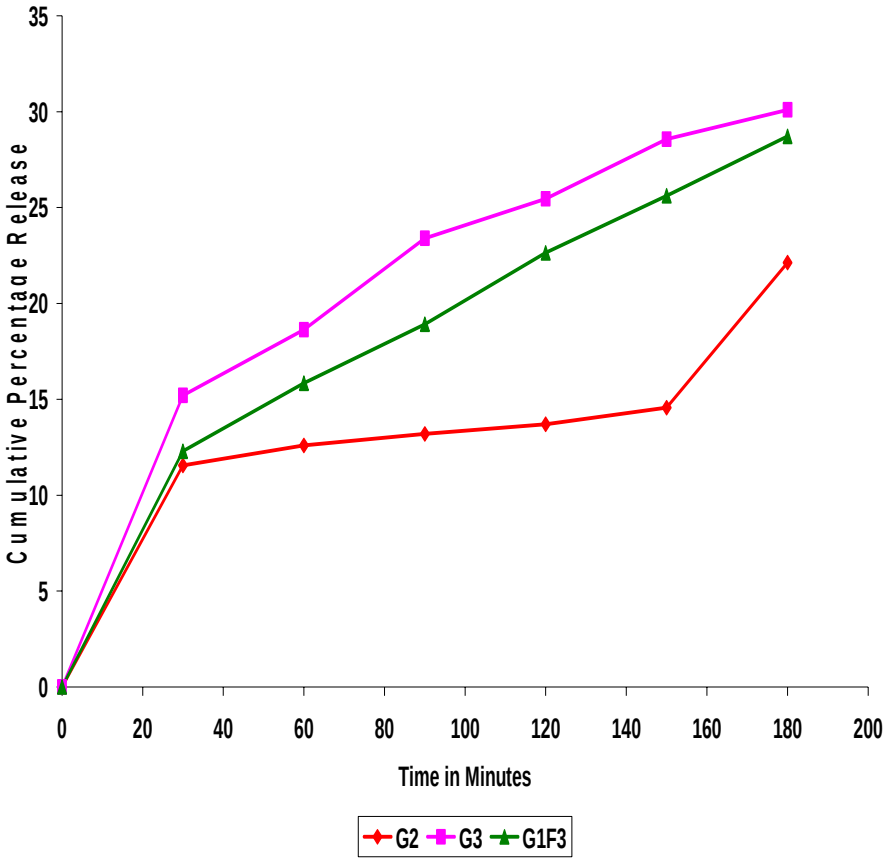


Table 11: Cumulative Percent Drug release from Indomethacin matrix tablets containing various concentrations of TSP (30- 70%) + Eudragit S100 (30%) in pH 6.8 buffer

TIME IN Hrs	PERCENTAGE RELEASE OF INDOMETHACIN (%)				
	G1F1	G1F2	G1F3	G1F4	G1F5
1	8.10	13.40	20.10	12.53	15.93
2	12.50	18.90	25.25	17.89	19.20
3	20.30	25.30	35.95	24.81	25.75
4	28.90	32.30	52.34	29.52	30.93
5	35.20	38.50	55.12	36.89	38.43
6	38.40	45.50	59.73	42.23	44.33
7	40.21	50.60	65.47	48.93	50.92
8	48.35	55.23	68.23	54.25	56.29
24	72.30	75.45	82.35	75.24	77.47

Fig 7: Comparison of release profiles of Indomethacin from matrix formulations containing varying concentrations of TSP (30-50%) + Eudragit S100 (30%) in pH 6.8 phosphate buffer

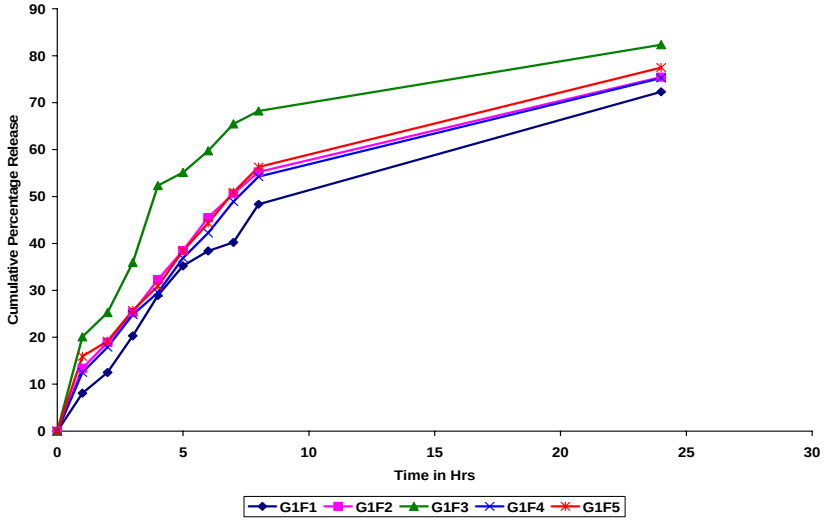


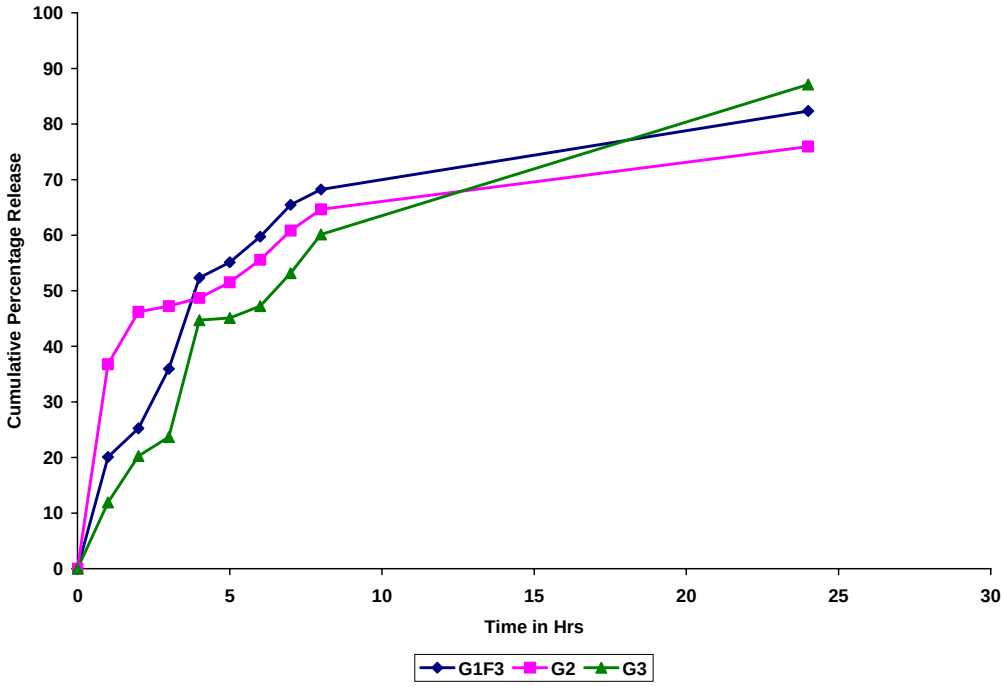
Table 12: Cumulative percent drug release from Indomethacin tablets containing Guar gum (50 %) + Eudragit S100 (30%) in pH 6.8 Buffer

Time in hrs	Percentage release of Indomethacin from G2 (%)
1	36.8
2	46.20
3	47.23
4	48.72
5	51.52
6	55.56
7	60.82
8	64.65
24	75.95

Table 13: Cumulative percent drug release from Indomethacin tablets containing Guar gum (50%) + Pectin (35%) + Eudragit S100 (30%) in pH 6.8 Buffer

Time in hrs	Percentage release of indomethacin from G3 (%)
1	11.9
2	20.26
3	23.70
4	44.72
5	45.11
6	47.23
7	53.18
8	60.14
24	87.11

Fig 8: Comparison of release profiles of Indomethacin from matrix formulations G2, G3, and G1F3 in pH 6.8 phosphate buffer



CONCLUSION

The present study was carried out to develop colon targeted drug delivery system for Indomethacin using a combination of Tamarind seed polysaccharide (TSP) and Eudragit S100 as carriers. Matrix tablets containing varying concentration (30%- 70%) of TSP along with 30% Eudragit S100 were prepared by wet granulation method and subjected to in vitro drug release studies. Matrix tablets containing 50% TSP and 30% Eudragit S100 was found to release the maximum amount of drug (82.35%) after 24 hour of dissolution study. Drug release achieved from matrix tablets containing a) Guar gum (50%) + Edragit S100 (30%) and b) Guar gum (50%) + Pectin (35%) + Eudragit S100(30%) was 75.9% and 87.1% respectively. Though there was statistically significant difference in the amount of drug released from these formulations, a combination of TSP and Eudragit S100 may be considered as a potential candidate for targeting Indomethacin to colon.

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